10/820,200

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     (FILE 'HOME' ENTERED AT 09:28:24 ON 15 AUG 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:29:08 ON 15 AUG 2006
L1         54958 S ALPHA (W)AMYLASE?
L2        369714 S FUNGAL OR FUNGAMYL
L3           605 S L1 (A)L2
L4           249 S TERMOSTAB?
L5             0 S L3 AND L4
L6         72916 S THERMOSTAB?
L7            39 S L3 AND L6
L8            20 DUP REM L7 (19 DUPLICATES REMOVED)
                 E BISGARD H/
                 E FRANTZEN H/AU
                 E BISGARD F H/
                 E SVCENSEN A/AU
                 E SVENDSEN A/AU
L9           425 S E3
                 E PEDERSEN S/AU
L10         1413 S E3
L11         1836 S L9 OR L10
L12            7 S L3 AND L11
L13            4 DUP REM L12 (3 DUPLICATES REMOVED)
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 NEWS  6  MAY 11  KOREAPAT updates resume
 NEWS  7  MAY 19  Derwent World Patents Index to be reloaded and enhanced
 NEWS  8  MAY 30  IPC 8 Rolled-up Core codes added to CA/CAplus and
                  USPATFULL/USPAT2
 NEWS  9  MAY 30  The F-Term thesaurus is now available in CA/CAplus
 NEWS 10  JUN 02  The first reclassification of IPC codes now complete in
                  INPADOC
 NEWS 11  JUN 26  TULSA/TULSA2 reloaded and enhanced with new search and
                  and display fields
 NEWS 12  JUN 28  Price changes in full-text patent databases EPFULL and PCTFULL
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 NEWS 14  JUl 14  FSTA enhanced with Japanese patents
 NEWS 15  JUl 19  Coverage of Research Disclosure reinstated in DWPI
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               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
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FILE 'EMBASE' ENTERED AT 09:29:08 ON 15 AUG 2006

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FILE 'HCAPLUS' ENTERED AT 09:29:08 ON 15 AUG 2006

FILE 'NTIS' ENTERED AT 09:29:08 ON 15 AUG 2006

FILE 'LIFESCI' ENTERED AT 09:29:08 ON 15 AUG 2006


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=> s alpha (w)amylase?
L1        54958 ALPHA (W) AMYLASE?

=> s fungal or fungamyl
L2       369714 FUNGAL OR FUNGAMYL

=> s l1 (a)l2
L3          605 L1 (A) L2

=> s termostab?
L4          249 TERMOSTAB?

=> s l3 and l4
L5            0 L3 AND L4

=> s thermostab?
L6        72916 THERMOSTAB?

=> s l3 and l6
L7           39 L3 AND L6

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8           20 DUP REM L7 (19 DUPLICATES REMOVED)

=> d 1-20 ibib ab

L8   ANSWER 1 OF 20     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:   2005346848     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 15998153
TITLE:              Impact of Fusarium culmorum on the polysaccharides of wheat
                    flour.
AUTHOR:             Wang Jinhua; Pawelzik Elke; Weinert Joachim; Wolf Gerhard A
CORPORATE SOURCE:   Institute of Agricultural Chemistry, University of
                    Gottingen, Carl-Sprengel-Weg 1, D-37075 Gottingen, Germany.
SOURCE:             Journal of agricultural and food chemistry, (2005 Jul 13)
                    Vol. 53, No. 14, pp. 5818-23.
```

Journal code: 0374755. ISSN: 0021-8561.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 7 Jul 2005
Last Updated on STN: 18 Aug 2005
Entered Medline: 17 Aug 2005

AB To assess the effects of Fusarium infection on the polysaccharides of winter wheat grain (Triticum aestivum L.), grain samples obtained from plants artificially inoculated with Fusarium culmorum were analyzed. Microscopy revealed obvious damage to the starch granules in the seriously infected samples. The Fusarium infection had no analytically detectable influence on the starch and total insoluble dietary fiber content of the wheat grain. There were significantly positive relationships between alpha-amylase activity, cellulase activity, total soluble dietary fiber content, pentosan content, and degree of infection quantified by an enzyme-linked immunosorbant assay, which would indicate the importance of fungal enzymes. A distinct higher Hagberg falling number (FN) was determined in the seriously infected samples, while the viscosity and sucrose content of the flour decreased. However, the addition of a liquid medium contaminated with F. culmorum led to a significant decrease in the FN. Depending on the type of buffer used, the alpha-amylase of F. culmorum demonstrated its maximum activity between pH 5.5 and pH 7.0 at 30-50 degrees C. Remarkably, this fungal alpha-amylase showed a thermostable characteristic and was active over a wide range of temperatures, from 10 to 100 degrees C. This type of thermostability suggests that the alpha-amylase of F. culmorum may damage starch granules throughout the processing of wheat flour, thereby inducing weak dough properties and unsatisfactory bread quality.

L8 ANSWER 2 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:997295 HCAPLUS
DOCUMENT NUMBER: 141:102002
TITLE: Heat inactivation of Aspergillus oryzae α-amylase at high and reduced water content
AUTHOR(S): Samborska, K.; Guiavarc'h, Y.; Van Loey, A.; Hendrickx, M.
CORPORATE SOURCE: Laboratory of Food Technology, Department of Food and Microbial Technology, Katholieke Universiteit Leuven, Heverlee, B-3001, Belg.
SOURCE: Mededelingen - Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen (Universiteit Gent) (2003), 68(3), 247-250
CODEN: MFLBER; ISSN: 1373-7503
PUBLISHER: Universiteit Gent, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The influence of water content on the kinetic parameters of heat inactivation of Aspergillus oryzae α-amylase was studied. Isothermal inactivation kinetics of Aspergillus oryzae α-amylase in both systems followed a first-order model. The influence of water content on the thermal stability of α-amylase was found to be significant. α-Amylase in maltodextrin system at reduced moisture content was much more thermostable than in solution The temperature range of inactivation in the reduced water content system was 100-115° compared to 62.5-70° for inactivation in aqueous solution The decrease of water content had also a significant effect on the z-value for thermal inactivation of Aspergillus oryzae α-amylase.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17360 BIOTECHDS
TITLE: Producing ethanol from starch-containing material e.g., tubers, roots, whole grain, for use in fuel, by fermentation comprises carrying out a secondary liquefaction step in the presence of a thermostable acid alpha-amylase;
alcohol preparation by bacterium or fungus fermentation and enzyme-catalyzed reaction
AUTHOR: VEIT C; FELBY C; FUGLSANG C C
PATENT ASSIGNEE: NOVOZYMES AS; NOVOZYMES NORTH AMERICA INC
PATENT INFO: WO 2002038787 16 May 2002
APPLICATION INFO: WO 2000-DK737 10 Nov 2000
PRIORITY INFO: US 2000-256015 15 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-479793 [51]
AB DERWENT ABSTRACT:

NOVELTY - Producing (M1) ethanol from starch-containing material, by fermentation involves carrying out a secondary liquefaction step in the presence of a thermostable acid alpha-amylase.

DETAILED DESCRIPTION - Producing (M1) ethanol from starch-containing material comprises: (a) liquefaction of starch-containing material in the presence of an alpha-amylase; (b) jet cooking; (c) liquefaction in the presence of a thermostable acid alpha-amylase; (d) saccharification; and (e) fermentation to produce ethanol, where steps (a)-(d) are performed in the order (a), (b), (c), (d) and step (e) is performed simultaneously to or following step (d). The method further involves carrying out a secondary liquefaction step in the presence of a thermostable acid alpha-amylase. INDEPENDENT CLAIMS are also included for the following: (1) a yeast (I) genetically modified to express thermostable maltogenic acid alpha-amylase having an amino acid sequence that is 70 % (more preferably 99 %) identical to a sequence of 484 amino acids, given in specification; and (2) use of a thermostable (maltogenic) acid alpha-amylase in the secondary liquefaction step in a process for production of ethanol.

BIOTECHNOLOGY - Preferred Method: (M1) further involves recovering ethanol, and a pre-saccharification step that is performed after the secondary liquefaction step (c) and before step (d). The starch-containing material is chosen from tubers, roots and whole grain (e.g., corn, wheat or barley or their combination) or combination of the materials. The starch-containing material is obtained from cereals, or is corns, cobs, wheat, barley, rye, milo and potatoes or their combination. The starch-containing material is preferably whole grain obtainable by process comprising milling of whole grain, where (M1) further comprises prior to step (a), the steps of: (i) milling (wet milling or dry milling) of whole grain; and (ii) forming a slurry comprising the milled grain and water to obtain the starch containing material. Optionally, the starch-containing material is a side stream from starch processing. (M1) further involves (f) distillation to obtain ethanol, where the fermentation in step (e) and the distillation in step (f) is carried out simultaneously or separately sequential, optionally followed by one or more process steps for further refinement of the ethanol. When the starch-containing material is milled whole grain, (M1) further involves (g) separation of whole stillage produced by the distillation in step (f), into wet grain and thin stillage and (h) recycling thin stillage to the starch-containing material prior to step (a). The fermentation step is performed using a microorganism such as bacteria and fungi e.g., Zymomonas sp. or Saccharomyces sp.. Preferably, the microorganism is capable of fermenting sugars to ethanol, and is yeast e.g., S. cerevisiae. The fermentation step is carried out in the presence of glucoamylase and/or protease e.g. fungal protease such as an acid fungal protease derived from a strain of Aspergillus preferably, A. niger. Optionally the protease is a neutral or alkaline protease such as a

protease derived from a strain of Bacillus. The method further involves adding a glucoamylase and/or phytase in order to promote the fermentation. Preferably, the phytase is microbial e.g., derived from Peniphra lycii or Aspergillus oryaze. The glucoamylase is also microbial, such as e.g., derived from a strain of A. niger or Talaromuces emersonii. The alpha-amylase employed in (M1), is microbial such as e.g., bacterial alpha-amylase, including e.g., a thermostable bacterial alpha-amylase derived from a genus of Bacillus, or is a thermostable fungal alpha-amylase derived from a strain of a genus Aspergillus, preferably A. niger. The liquefied material in step (c) has been liquefied to a dextrose equivalent (DE) of 5 - 15, e.g., 8 - 12, such as e.g., 10 - 12, where prior to step (a), the steps of: (i) milling of whole grain; and (ii) forming a slurry comprising the milled grain and water, are carried out. The liquefaction step is performed at 60 - 95 degrees Centigrade for 10 - 120 min, (preferably at 75 - 90 degrees Centigrade, more preferably at 75 - 80 degrees Centigrade) for 15 - 40 min (15 - 80 min). In step (c), in addition to the thermostable acid alpha-amylase (preferably, thermostable maltogenic acid alpha-amylase) is also added an alpha-amylase which is not a thermostable acid alpha-amylase, i.e., e.g., the alpha-amylase TTC. The thermostable maltogenic acid alpha-amylase is an alpha-amylase having an amino acid sequence which has 70 % (most preferably 99 %) identity to a sequence of 484 amino acids (S1), given in specification. More preferably, the thermostable maltogenic alpha-amylase is an alpha-amylase having a sequence of (S1). The maltogenic alpha-amylase enzyme is such that when using DE 12 alpha-amylase TTC liquefied corn starch at 30 % dry substance at 60 degrees Centigrade, pH 4.5 and dosing the enzyme at 1 acid fungal alpha-amylase unit (AFAU)/g dry substance, the enzyme will in 24 hours catalyze the formation of 15 % (more preferably 30 %) w/w maltose as based on the total amount of starch. The thermostable alpha-amylase maintains more than 90 % of its activity for 1 hour at 70 degrees Centigrade using a DE 12 alpha-amylase TTC liquefied corn starch at 30 % dry substance as substrate, pH 5.5, 0.1 M citrate buffer and 4.3 mM Ca2+, and maintains more than 80 % of its activity for 15 minutes in the range of 50 - 80 degrees Centigrade using a DE 12 alpha-amylase TTC liquefied corn starch at 30 % dry substance as substrate. The maltogenic thermostable acid alpha-amylase used in the secondary liquefaction step is acid such that enzyme maintains more than 70 % of its activity in the range of pH 3.5 - 5 (e.g., at pH 4) at the conditions: substrate DE 12 alpha-amylase TTC liquefied corn starch at 30 % dry substance, temperature 40 degrees Centigrade and 0.1 M citrate buffer and 4.3 mM Ca2+. The secondary liquefaction step and the fermentation step is performed essentially simultaneously and where the alpha-amylase for the secondary liquefaction is expressed by the microorganism, preferably yeast, the microorganism being genetically modified so as to express the alpha-amylase. (M1) further involves adding one or more additional enzymes in one or more of the process steps. The additional enzyme is not an alpha-amylase, preferably a thermostable maltogenic acid alpha-amylase. All process steps in (M1) are performed batch wise, or where all process steps are performed as a continuous flow, or where one or more process step(s) is(are) performed batch wise and one or more process step(s) is(are) performed as a continuous flow.

USE - The method is used in producing ethanol from a starch-containing material such as tubers, roots or whole grain (e.g., corn, wheat or barley or their combination) or combination of the materials. Preferably, ethanol is produced from starch-containing material that is obtained from cereals or from corns, cobs, wheat, barley, rye, milo and potatoes or their combination. The ethanol produced by above mentioned method is used as fuel alcohol and/or fuel additive (all claimed). The ethanol is also useful as drinking ethanol i.e., potable neutral spirits or industrial ethanol.

ADVANTAGE - By employing the thermostable acid

alpha-amylase of the secondary liquefaction step, the method provides an improved process of producing ethanol. Overall yield and/or process economy is increased, and the fermentation time is lowered. Further the process enhances the fermentation efficiency, e.g., by reducing the residual starch otherwise left over in the fermentation, and reduces or eliminates the need for a pre-saccharification step. By the process overall yield and/or process economy is increased. The thermostable maltogenic acid alpha-amylase will, when used in secondary liquefaction, produce a higher number of fermentable sugars (maltose) as compared to the non-maltogenic alpha-amylase presently employed. This reduces the fermentation time and/or the dosage of glucoamylase enzyme which is required to form fermentable sugars. Also as molecules of lower molecular weight are formed the viscosity will be reduced as compared to non-maltogenic alpha-amylase. Furthermore, the thermostable maltogenic acid alpha-amylase by being active during fermentation conditions, and since this enzyme has an endo-breakdown mechanism it will in combination with glucoamylase which is an exoenzyme enables a more efficient hydrolysis during fermentation. The method also reduces or eliminates need for pre-saccharification step.

EXAMPLE - Four hundred mL of a ground corn slurry was liquefied by a bacterial alpha-amylase and jet cooked at 105 degrees Centigrade for 5 min, the resulting corn mash had 30 % dry substance, dextrose equivalent (DE) 7, and pH 5. The mash was heated to 80 degrees Centigrade and the viscosity was measured to 500 centipoise (CPS) using a VT 180 viscosimeter. The mash was treated with a thermostable acidic alpha-amylase from Aspergillus niger. The enzyme loading was 0.25 acid fungal alpha-amylase unit (AFAU)/g of dry matter, with 1 AFAU defined as the amount of enzyme that under standard conditions (37 degrees Centigrade, pH 2.5 in 0.01 M acetate buffer) hydrolyzes 5.25 g starch so that the hydrolyzed starch is only slightly colored by addition of iodine-potassium-iodide. After 30 min the viscosity and DE value were measured to 350 CPS and DE 12, which showed that a final liquefaction of the corn mash was obtained. (33 pages)

L8 ANSWER 4 OF 20 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002616980 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12374409
TITLE: Measurement of alpha-amylase activity in white wheat flour, milled malt, and microbial enzyme preparations, using the Ceralpha assay: collaborative study.
AUTHOR: McCleary Barry V; McNally Marian; Monaghan Dympna; Mugford David Cbarry@megazyme.com
SOURCE: Journal of AOAC International, (2002 Sep-Oct) Vol. 85, No. 5, pp. 1096-102.
Journal code: 9215446. ISSN: 1060-3271.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200304
ENTRY DATE: Entered STN: 11 Oct 2002
Last Updated on STN: 2 Apr 2003
Entered Medline: 1 Apr 2003

AB This study was conducted to evaluate the method performance of a rapid procedure for the measurement of alpha-amylase activity in flours and microbial enzyme preparations. Samples were milled (if necessary) to pass a 0.5 mm sieve and then extracted with a buffer/salt solution, and the extracts were clarified and diluted. Aliquots of diluted extract (containing alpha-amylase) were incubated with substrate mixture under defined conditions of pH, temperature, and time. The substrate used was nonreducing end-blocked p-nitrophenyl maltoheptaoside (BPNPG7) in the presence of excess quantities of thermostable alpha-glucosidase. The blocking group in BPNPG7 prevents hydrolysis of this substrate by exo-acting enzymes such as amyloglucosidase, alpha-glucosidase, and

beta-amylase. When the substrate is cleaved by endo-acting alpha-amylase, the nitrophenyl oligosaccharide is immediately and completely hydrolyzed to p-nitrophenol and free glucose by the excess quantities of alpha-glucosidase present in the substrate mixture. The reaction is terminated, and the phenolate color developed by the addition of an alkaline solution is measured at 400 nm. Amylase activity is expressed in terms of Ceralpha units; 1 unit is defined as the amount of enzyme required to release 1 micromol p-nitrophenyl (in the presence of excess quantities of alpha-glucosidase) in 1 min at 40 degrees C. In the present study, 15 laboratories analyzed 16 samples as blind duplicates. The analyzed samples were white wheat flour, white wheat flour to which fungal alpha-amylase had been added, milled malt, and fungal and bacterial enzyme preparations. Repeatability relative standard deviations ranged from 1.4 to 14.4%, and reproducibility relative standard deviations ranged from 5.0 to 16.7%.

L8 ANSWER 5 OF 20 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 3
ACCESSION NUMBER: 1998:301219 BIOSIS
DOCUMENT NUMBER: PREV199800301219
TITLE: Thermostability and browning development of fungal alpha-amylase freeze-dried in carbohydrate and PVP matrices.
AUTHOR(S): Terebiznik, M. R.; Buera, M. P.; Pilosof, A. M. R. [Reprint author]
CORPORATE SOURCE: Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
SOURCE: Lebensmittel-Wissenschaft and Technologie, (1998) Vol. 31, No. 2, pp. 143-149. print.
CODEN: LBWTAP. ISSN: 0023-6438.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Jul 1998
Last Updated on STN: 15 Jul 1998

AB Thermal stability and browning development of systems containing fungal alpha-amylase in lactose, raffinose, sucrose, trehalose and polyvinylpyrrolidone (PVP) matrices after heat treatment at 70degreeC in a constant relative humidity (RH) environment and in connection with phase transitions were studied. Matrices showed considerable variability in their ability to stabilize alpha-amylase and in browning development. Amorphous trehalose was the most efficient matrix for preventing non-enzymatic browning and thermal inactivation of the alpha-amylase. Remaining alpha-amylase activity decreased as RH% and heating time were increased, the extent of the effect being different for each matrix. Trehalose matrix appeared the most efficient in preventing alpha-amylase deactivation at '0', 11 and 20% RH. At 42% RH all the matrices showed the lowest degree of enzyme stabilization. The matrices' glassy condition was not enough to ensure enzyme thermal stability; the glassy matrices of trehalose and lactose allowed the retention of 80% enzyme activity after 96 h of heat treatment; the remaining activity in raffinose and PVP matrices was 50% or less. The degree of enzymatic activity protection given by different glassy matrices was related to their molecular weight (which affects molecular packing) and to their associated water content. The degree of browning in each matrix did not follow the same pattern as loss of enzymatic activity. Browning in trehalose and PVP systems was minimal and not accelerated above the glass transition, even in conditions at which trehalose crystallization should occur (42% RH).

L8 ANSWER 6 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1995-12712 BIOTECHDS
TITLE: Enzymes used in limit conditions. Examples in food technology;

e.g. starch saccharification using thermostable alpha-amylase from Bacillus licheniformis, and bitterness removal from tenderized meat using protease

AUTHOR: Nicolas J
CORPORATE SOURCE: Nat.Coll.Arts+Ind.Paris
LOCATION: Biochimie Industrielle et Agroalimentaire, 292, rue Saint-Martin, 75141 Paris Cedex 03, France.
SOURCE: C.R.Acad.Agric.Fr.; (1995) 81, 2, 11-17
CODEN: CRAFEQ
ISSN: 0989-6988
DOCUMENT TYPE: Journal
LANGUAGE: French

AB Normally for saccharification, starch undergoes 2 processes of gelatinization at 105-110 deg then amylolysis with enzymes at a maximum of 40 deg. Although some fungal alpha-amylases (EC-3.2.1.1) have an optimum at 50 deg, a thermostable enzyme from Bacillus licheniformis acted above 90 deg and at high enzyme concentrations up to 110 deg. This alpha-amylase allowed gelatinization and enzymic hydrolysis in a single rapid step initially at 105 deg then at 95 deg. Treatment of proteins with proteolytic enzymes to tenderize meat and to produce partial hydrolysis of plant and fish proteins can yield bitter-tasting peptides or hydrophobic residues. Proteases were used to remove undesirable materials. Following initial hydrolysis with 1 enzyme, the hydrolyzate was concentrated to a level of 30-40% peptides, then the same or a different protease was added and the pH adjusted to 1, favoring biosynthesis, to link the peptides to protein chains, thereby eliminating undesirable peptides. (22 ref)

L8 ANSWER 7 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:579286 HCAPLUS
DOCUMENT NUMBER: 119:179286
TITLE: Examination in starch degradation using technical enzyme preparations in bioethanol production
AUTHOR(S): Senn, T.
CORPORATE SOURCE: Inst. Lebensmitteltechnol., Univ. Hohenheim, Stuttgart, W-7000/70, Germany
SOURCE: DECHEMA Biotechnology Conferences (1992), 5(Pt. A, Microbial Principles in Bioprocesses: Cell Culture Technology, Downstream Processing and Recovery), 155-60
CODEN: DBCOEU; ISSN: 0934-3792
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The degradation of starch in corn mashes using several tech. enzyme prepns., alone or in combination with others, was studied. The following enzymes were used: thermostable α-amylase from Bacillus licheniformis, fungal .alpha.-amylase from Aspergillus oryzae, glucoamylase from Rhizopus and A. niger, and dried barley malt.

L8 ANSWER 8 OF 20 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 1992:95362 BIOSIS
DOCUMENT NUMBER: PREV199293051912; BA93:51912
TITLE: AUTOMATED ENZYMIC DETERMINATION OF STARCH BY FLOW INJECTION ANALYSIS.
AUTHOR(S): KARKALAS J [Reprint author]
CORPORATE SOURCE: UNIVERSITY OF STRATHCLYDE, DEP BIOSCIENCE AND BIOTECHNOL, 131 ALBION ST, GLASGOW G1 1SD, UK
SOURCE: Journal of Cereal Science, (1991) Vol. 14, No. 3, pp. 279-286.
CODEN: JCSCDA. ISSN: 0733-5210.
DOCUMENT TYPE: Article
FILE SEGMENT: BA

LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 12 Feb 1992
Last Updated on STN: 14 Apr 1992

AB A method is described for the continuous enzymic determination of starch by unsegmented flow injection analysis (FIA). Pre-weighed starch-containing samples were treated with thermostable bacterial alpha-amylase for 10 min at 100° C, followed by filtration and loading of the filtrates onto an autosampler. The filtrates were then injected sequentially into the calibrated FIA apparatus, and hydrolysed continuously with fungal amyloglucosidase at pHh 4.5 and 60° C. The resulting glucose was measured colorimetrically at 505 nm by the continuous addition of a reagent stream containing glucose oxidase, peroxidase and a chromogen that gives a pink colour. The system's throughput is 32 samples per hour and the consumption of reagents was low in comparison with manual methods.

L8 ANSWER 9 OF 20 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 4
ACCESSION NUMBER: 1989:492065 BIOSIS
DOCUMENT NUMBER: PREV198988118602; BA88:118602
TITLE: EFFECT OF POLYOLS ON FUNGAL ALPHA AMYLASE THERMOSTABILITY.
AUTHOR(S): GRABER M [Reprint author]; COMBES D
CORPORATE SOURCE: DEP DE GENIE BIOCHMIQUE ET ALIMENTAIRE, UA-CNRS-544, INST NATIONAL DES SCI APPLIQUEES, AVE DE RANGUEIL, 31077 TOULOUSE CEDEX, FRANCE
SOURCE: Enzyme and Microbial Technology, (1989) Vol. 11, No. 10, pp. 673-677.
CODEN: EMTED2. ISSN: 0141-0229.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 2 Nov 1989
Last Updated on STN: 2 Nov 1989

AB The influence of different polyols (ethylene glycol, glycerol, erythritol, xylitol, and sorbitol) on the thermostability of fungal alpha-amylase at 60° C has been studied. The results obtained show a stabilizing effect in the presence of polyols. In the case of 4 M sorbitol solution, the enzyme half-life is 2000-fold longer than in pure water. These polyols have been found as competitive inhibitors for alpha-amylase and their stabilizing effect has been correlated to their affinity constant except for sorbitol. The influence of two polyol isomers (arabitol and mannitol) on activity and stability of alpha-amylase has also been investigated.

L8 ANSWER 10 OF 20 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 90143168 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2515679
TITLE: Physico-chemical properties of Aspergillus flavus var. columnaris alpha-amylase.
AUTHOR: Ali F S; Abdel-Moneim A A
CORPORATE SOURCE: Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, Egypt.
SOURCE: Zentralblatt fur Mikrobiologie, (1989) Vol. 144, No. 8, pp. 615-21.
Journal code: 8209932. ISSN: 0232-4393.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 28 Mar 1990
Last Updated on STN: 28 Mar 1990
Entered Medline: 15 Mar 1990

AB The best temperature for the preservation of A. flavus var. columnaris alpha-amylase was -5 degrees C followed by 5 degrees C. CaCl2 at 0.005 M had no effect on the activity in both temperatures. Repeated freezing (-5 degrees C) and thawing followed by freezing (-5 degrees C) had no effect on stability of alpha-amylase. On the other hand, 25 degrees C was the lowest preservation temperature without any effect on the stability on alpha-amylase. 0.005 M CaCl2 decreased the activity of alpha-amylase and reached a 100% inhibition at 35th day. The fungal alpha -amylase had an optimum temperature of 55 degrees C at pH 4.6, but had 60 degrees C in buffer containing 0.005 M CaCl2 and 50 degrees C in buffer containing 0.005 M Na2-EDTA. The addition of 0.01 M CaCl2 greatly increased the thermostability of alpha-amylase at 40, 45, 50, 55 and 60 degrees C for 30 min. Optimum pH for alpha-amylase only was 5, but in the presence of 0.01 M CaCl2 or Na2-EDTA 5.6. The enzyme only was stable for 4 h at 25 degrees C. Whereas addition of 0.01 M CaCl2 showed a loss of 4% compared to a 22% loss in the presence of 0.01 M Na2-EDTA after 4 h at 25 degrees C and 65% loss in the presence of 0.01 M CaCl2 together with 0.01 M Na2-EDTA in the beginning and a 100% loss after 4 h at 25 degrees C. The optimum temperature for the activity of alpha-amylase at pH 5 was 50 degrees C for the enzyme only but 55 degrees C in the presence of 0.01 M CaCl2. However, at pH 6 and 7 optimum temperature was 55 degrees C for the activity of the enzyme only or with 0.01 M CaCl2. The presence of 0.01 M CaCl2 at pH 5, 6 and 7 resulted in increase of enzyme activity at the temperatures above 50, 40 and 25 degrees C, respectively. However, 0.01 M CaCl2 at pH 5 and 6 resulted in decreasing enzyme activity at temperatures below 55 and 45 degrees C, respectively.

L8 ANSWER 11 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-05360 BIOTECHDS
TITLE: Enzymatic processing of starch in food processing; liquefaction, saccharification, isomerization using alpha-amylase, glucoamylase, beta-amylase, glucose-isomerase for e.g. production of high fructose syrup; review (conference paper)
AUTHOR: Richter G; Tegge G
CORPORATE SOURCE: Miles-Kali-Chemie
LOCATION: Miles Kali-Chemie Gmbh & Co. KG, Hannover, Germany.
SOURCE: Biotechnol.Food.Ind.; (1988) 401-23
DOCUMENT TYPE: Journal
LANGUAGE: English
AB During the past 3 decades, partial or complete starch hydrolysis has moved from the use of acid to enzymes, mainly from microbial sources. Enzymatic processes in starch conversion were described, including high fructose syrup production by starch liquefaction with bacterial alpha-amylase (EC-3.2.1.1, saccharification with fungal glucoamylase (EC-3.2.1.3) and partial isomerization of D-glucose by glucose-isomerase (EC-5.3.1.5). The use of enzymes rather than acid facilitates the production of syrups of high standards from low quality raw materials. Liquefaction was discussed in relation to the need for a thermostable alpha-amylase e.g. Bacillus licheniformis OPTITHERM (optimum temperature 88-90 deg). Fungal alpha-amylase and glucoamylase (e.g. Aspergillus niger OPTIDEX), and cereal beta-amylase (EC-3.2.1.2) are the most important saccharifying enzymes. In contrast to liquefaction and saccharification which are run continuously, glucose isomerization to fructose uses immobilized glucose-isomerase (e.g. Streptomyces rubiginosus OPTISWEET). Some specific examples of industrial applications are described. (4 ref)

L8 ANSWER 12 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1986-10226 BIOTECHDS
TITLE: Studies on the application of maltogenic amylase in the production of maltose containing syrup; use in combination with pullulanase and fungal

alpha-amylase
AUTHOR: Slominska L; Starogardzka G
LOCATION: Central Laboratorium Przemyslu Ziemniaczanego, Zwierzniecka 18, 60-814 Poznan, Poland.
SOURCE: Starch; (1986) 38, 6, 205-10
CODEN: STARDD
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The thermostable and relatively acid stable maltogenic amylase produced by Bacillus stearothermophilus was studied, during an analysis of the advantages of using a maltogenic amylase for maltose production during saccharification. Experiments were performed using B. stearothermophilus maltogenic amylase SP 295, with Polish potato starch as the substrate. A slurry of the starch was subjected to liquefaction at 85 deg for 1 hr with Bacillus subtilis alpha-amylase (EC-3.2.1.1) (Amylogal CS). The pH was adjusted to 5.0-5.3 and the temperature raised to 105 deg for 15-30 min. Spray-dried maltodextrin was redissolved and saccharified using maltogenic amylase, Bacillus sp. pullulanase (EC-3.2.1.41) and fungal (Aspergillus oryzae) alpha-amylase at 60 deg for 72 hr. With the maltogenic amylase, potato syrup containing 70-80% maltose was obtained from DE 12 enzyme liquefied starch at a concentration of 30-35%. A combination of the 3 saccharifying enzymes gave 85% maltose. Maltogenic amylase used with pullulanase increased the maltose yield and decreased saccharification time. (10 ref)

L8 ANSWER 13 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1979:401926 HCAPLUS
DOCUMENT NUMBER: 91:1926
TITLE: The influence of charged matrix surfaces on the thermostabilizing effect of calcium ions on immobilized fungal .alpha.-amylase
AUTHOR(S): Fischer, J.; Ulbrich, R.; Schellenberger, A.
CORPORATE SOURCE: Inst. Enzymol. Tech. Mikrobiol., Berlin, 104, Ger. Dem. Rep.
SOURCE: Acta Biologica et Medica Germanica (1979), 37(9), 1413-24
CODEN: ABMGAJ; ISSN: 0001-5318
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The stabilizing effect of Ca2+ on fungal .alpha.-amylase immobilized on a polystyrene anion exchanger (P+-amylase) was investigated and compared to the behavior of soluble amylase. Moreover, γ-(1,4-benzoquinone-2-yl)-aminopropyl silica-amylase as a conjugate with weakly basic amino groups and γ-succinamidopropyl silica-amylase (Si--amylase) as a conjugate with free carboxyl groups were applied for comparison. Depending on the Ca2+ concentration, the immobilized amylases showed a lower thermal stability than the soluble enzyme. The reduced stability was attributed to matrix effects in the microenvironment of the immobilized amylases and the Ca2+ concentration in the carrier phase, which was changed in comparison with the external solution Contrary to the nonmeasurable matrix effects in the microenvironment, altered Ca2+ concns. in the carrier phase of the polystyrene anion exchanger (P+) and γ-succinamidopropyl silica (Si-) could be detected. With increasing Ca2+ concentration, a greater decrease of activity was observed for the soluble amylase than for the immobilized enzymes. The thermal stability of soluble amylase and P+-amylase was studied in dependence on pH. In the acidic pH-range, P+-amylase indicated a higher thermal stability than the soluble enzyme in the presence of Ca2+ as well as in the absence of Ca2+. Contrary to soluble amylase, the stabilizing effect of Ca2+ on P+/amylase already begins at pH 3.5. Kinetic investigations for thermal inactivation were performed on soluble amylase and P+-amylase in the presence and absence of Ca2+ in the temperature range between 44-60°. Thermal inactivation proceeded by

1st-order reactions. The inactivation rate consts. served as a measure of thermal stability for discussing the stabilizing effect by Ca2+ depending on the temperature The activation energies of inactivation were determined from the
Arrhenius plot of the inactivation rate consts.

L8 ANSWER 14 OF 20 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 79161711 MEDLINE
DOCUMENT NUMBER: PubMed ID: 749472
TITLE: The influence of charged matrix surfaces on the thermostabilizing effect of calcium ions on immobilized fungal alpha-amylase.
AUTHOR: Fischer J; Ulbrich R; Schellenberger A
SOURCE: Acta biologica et medica Germanica, (1978) Vol. 37, No. 9, pp. 1413-24.
Journal code: 0370276. ISSN: 0001-5318.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197906
ENTRY DATE: Entered STN: 15 Mar 1990
Last Updated on STN: 15 Mar 1990
Entered Medline: 11 Jun 1979

AB The stabilizing effect of calcium ions on fungal alpha -amylase (EC 3.2.1.1) immobilized on a polystyrene anion exchanger (P+ amylase) was investigated and compared to the behaviour of soluble amylase. Moreover, gamma-(1,4-benzoquinone-2-yl)-aminopropyl silica-amylase (Si(n) amylase) as a conjugate with weakly basic amino groups and gamma-succinamidopropyl silica amylase (Si- amylase) as a conjugate with free carboxyl groups were applied for comparison. Depending on the calcium ion concentration the immobilized amylases showed a lower thermal stability than the soluble enzyme. The reduced stability was attributed to matrix effects in the microenvironment of the immobilized amylases and the calcium ion concentration in the carrier phase, which was changed in comparison with the external solution. Contrary to the non-measurable matrix effects in the microenvironment, altered calcium ion concentrations in the carrier phase of the polystyrene anion exchanger (P+) and gamma-succinamidopropyl silica (Si-) could be detected. With increasing calcium ion concentration a greater decrease of activity was observed for the soluble amylase than for the immobilized enzymes. The thermal stability of soluble amylase and P+ amylase was studied in dependence on pH. In the acidic pH-range P+ amylase indicated a higher thermal stability than the soluble enzyme in the presence of Ca2+ as well as in the absence of Ca2+. Contrary to soluble amylase the stabilizing effect of calcium ions on P+ amylase begins already at pH 3.5. Kinetic investigations for thermal inactivation were performed on soluble amylase and P+ amylase in the presence and absence of Ca2+ in the temperature range between 44--60 degrees C. Thermal inactivation proceeded by first order reactions. The inactivation rate constants kin served as a measure of thermal stability for discussing the stabilizing effect by Ca2+ depending on the temperature. The activation energies of inactivation EA were determined from the Arrhenius-plot of the inactivation rate constants.

L8 ANSWER 15 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1973:417121 HCAPLUS
DOCUMENT NUMBER: 79:17121
TITLE: Influence of crust pigments in white baking
AUTHOR(S): Benedickt, G.
CORPORATE SOURCE: Kulmbach, Fed. Rep. Ger.
SOURCE: Ber. Tag. Baeckerei-Technol., Vortr. Tag. (1972), 79-89. Granum-Verlag: Detmold, Ger.

CODEN: 26RKAJ
DOCUMENT TYPE: Conference
LANGUAGE: German
AB The mixing of various substances into the dough had the following results. Fructose and glucose result in the highest degree of browning of the crust. Sucrose is just as effective if first hydrolyzed by invertase. Maltose and lactose lead to only .apprx.1/4 the browning caused by fructose and glucose. The browning effect of malt meal depends upon the enzymes in it. Fungal .alpha.-amylase causes much weaker browning than malt α-amylase, probably because of the low heat stability of the fungal amylase. Milk and plant protein prepns., and degraded starch have little browning effect, and fats have none.

L8 ANSWER 16 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1962:47719 HCAPLUS
DOCUMENT NUMBER: 56:47719
ORIGINAL REFERENCE NO.: 56:9085a-c
TITLE: A method for the determination of relative amounts of malted-wheat, fungal (Aspergillus oryzae) and bacterial (Bacillus subtilis) α-amylase in mixtures, and its application to malted wheat
AUTHOR(S): Fleming, James R.; Miller, Byron S.; Johnson, John A.
CORPORATE SOURCE: Kansas Agr. Expt. Sta., Manhattan
SOURCE: Cereal Chemistry (1961), 38, 479-86
CODEN: CECHAF; ISSN: 0009-0352
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB The method is based on differences of thermostability of the 3 types of α-amylases and the solution of 3 simultaneous equations of the form C + F + B = total activity, where C, F, and B represent the contribution of cereal (wheat malt), fungal, and bacterial α-amylases, resp., to the total activity of the mixture The coeffs. of C, F, and B are the % of the resp. α-amylases remaining after heating of buffered solns. of each enzyme sep. at the 3 temps. used in the determination Known mixts. of the 3 enzymes were analyzed by the method and most of the calculated values agreed within acceptable limits with the measured values. Coeffs. for the specific prepns. were 1, 1, 1 at 65°; 1, 0.54, 1 at 70°; and 0.58, 0.05, 1 at 75° for C, F, and B, resp. These coefficients might not be valid for other sources of α-amylases. The method was applied to malted wheats differing in moldiness. The amount of bacterial α-amylase was slight and the amount of fungal .alpha.-amylase increased with an increase in mold growth.

L8 ANSWER 17 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1957:58148 HCAPLUS
DOCUMENT NUMBER: 51:58148
ORIGINAL REFERENCE NO.: 51:10781b-d
TITLE: The soluble dextrin fraction and sugar content of bread baked with α-amylase from different sources
AUTHOR(S): Beck, Horst; Johnson, John A.; Miller, Byron S.
CORPORATE SOURCE: Lab. Central, Porto Alegre, Brazil
SOURCE: Cereal Chemistry (1957), 34, 211-17
CODEN: CECHAF; ISSN: 0009-0352
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB Soluble dexrin extracted from bread crumb 1 hr. after baking increased with each increment, 140,560, 1120 units of α-amylase, from com. malted wheat flour and with bacterial (7, 35, and 140) units of α-amylase. Fungal .alpha.-amylase did not affect the amount

of extracted soluble dextrins because of its low thermostability. The concentration of the malted wheat flour and fungal enzyme did not have an appreciable effect on the average chain length of the dexrin. The average chain length of the soluble dextrins extracted from bread crumb made with bacterial amylase was several times greater than that obtained from bread made with malted wheat flour and fungal enzymes. The residual glucose in bread crumb increased rapidly with increasing concns. of bacterial amylase but less rapidly with increasing concns. of malted wheat flour or lungal α-amylase. The residual maltose in bread crumb increased much less with increasing quantities of fungal than with comparable quantities of malted wheat flour or bacterial α-amylase.

L8 ANSWER 18 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1954:19673 HCAPLUS
DOCUMENT NUMBER: 48:19673
ORIGINAL REFERENCE NO.: 48:3580g-i,3581a-f
TITLE: The use of fungal enzymes for breadmaking purposes
AUTHOR(S): Greup, D. H.; Hintzer, H. M. R.
CORPORATE SOURCE: Central Instituut Voor Voedingsonderzoek T.N.O., Wageningen, The Netherlands
SOURCE: 2nd Intern. Congr. Fermentation Inds. Knocke, Lectures and Communs. (1952) 232-338
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB The need for maintaining an optimum concentration of α- and β-amylase in the process of breadmaking and the suitability of fungal enzyme prepns. for this purpose are discussed. α- and β-Amylases together act on starch and bring about a rapid saccharification which provides the fermentable sugar for the yeast. A deficiency of α-amylase limits saccharification and makes the gas production insufficient in the final stages. This deficiency of normal sound flour can be avoided by using flour from sprouted wheat, but owing to its excessive content of dextrins, this has the advantage of making the dough and bread crumbs sticky. The long-employed alternative is to supplement the flour with malt-enzyme prepns., but the use of enzyme prepns. from several molds, such as certain strains of Aspergillus oryzae, is recently receiving considerable interest. Some characteristic properties of the crystalline fungal . alpha.-amylase, prepared by fractionation with (NH4)2SO4, are lack of thermostability, stability in the cold between pH 4.7 and 7.8, isoelec. point at about 4.0, and nondependence on any ions such as Ca++ for its activity. The effect, on the quality of Dutch white bread, of the use of 2 fungal enzyme prepns., Diastase 33 (I) and Rhozyme-S (II), is studied, the former being highly amylolytic and poorly proteolytic while the latter is a highly amylolytic and a highly proteolytic preparation The results showed that these prepns. when used at suitable levels improved the quality of the bread, while excessive use was detrimental. The results from baking tests were: (1) Dough consistency appeared to decrease and dough-handling properties improved. This effect was greater in the case of II, since for I the amount of susceptible starch was a limiting factor, while II was not limited by the nature of the gluten substrate. (2) Bread properties such as the color of the crust, loaf volume, and crumb characteristics improved. Crumb compressibility at different storage times was determined by using a panimeter and this showed that softness of the bread had increased. Similarly carried out studies showed that, owing to the effect of I and II, the maltose value was raised only slightly while gas production, measured over a period of several hrs., was increased considerably, I being less effective than II. It is suggested that increased gas production, which becomes more pronounced under the action of heat during the first half of the baking process, contributes to better oven spring and improved loaf volume The maximum paste viscosity (measured with a Brabender Amylograph) was hardly affected by the fungal enzymes because of their low inactivation temps. Thus, it is claimed that treatment with fungal enzymes permits the formation of sugars

without any appreciable decrease in the viscosity of gelatinized starch. Also, the formation of dextrins at elevated temps. will be held at a min. and the choice of the enzyme level may be less critical than for malt α-amylase, which has a relatively high inactivation temperature Other suggested advantages are increased availability and mild degradation conditions of starch and liberation of bound β-amylase, which increase the rate of starch hydrolysis and gas production. The presence of other factors not included in this study, e.g. the quality of susceptible starch, nature of starch granules and gluten proteins, α-amylase content of flour, influence of proteolytic enzymes on bound enzymes, etc. may, of course, influence the response of the flour to fungal enzymes.

L8 ANSWER 19 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1954:19674 HCAPLUS
DOCUMENT NUMBER: 48:19674
ORIGINAL REFERENCE NO.: 48:3580g-i,3581a-f
TITLE: The use of fungal enzymes for breadmaking purposes
AUTHOR(S): Greup, D. H.; Hintzer, H. M. R.
CORPORATE SOURCE: Central Instituut Voor Voedingsonderzoek T.N.O., Wageningen, Neth.
SOURCE: Central Inst. Voedingsonderzoek T.N.O. Afdel. Graan-, Meel-en Broodonderzoek Wageningen, Mededel (1952), No. 44E,
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB The need for maintaining an optimum concentration of α- and β-amylase in the process of breadmaking and the suitability of fungal enzyme prepns. for this purpose are discussed. α- and β-Amylases together act on starch and bring about a rapid saccharification which provides the fermentable sugar for the yeast. A deficiency of α-amylase limits saccharification and makes the gas production insufficient in the final stages. This deficiency of normal sound flour can be avoided by using flour from sprouted wheat, but owing to its excessive content of dextrins, this has the advantage of making the dough and bread crumbs sticky. The long-employed alternative is to supplement the flour with malt-enzyme prepns., but the use of enzyme prepns. from several molds, such as certain strains of Aspergillus oryzae, is recently receiving considerable interest. Some characteristic properties of the crystalline fungal . alpha.-amylase, prepared by fractionation with (NH4)2SO4, are lack of thermostability, stability in the cold between pH 4.7 and 7.8, isoelec. point at about 4.0, and nondependence on any ions such as Ca++ for its activity. The effect, on the quality of Dutch white bread, of the use of 2 fungal enzyme prepns., Diastase 33 (I) and Rhozyme-S (II), is studied, the former being highly amylolytic and poorly proteolytic while the latter is a highly amylolytic and a highly proteolytic preparation The results showed that these prepns. when used at suitable levels improved the quality of the bread, while excessive use was detrimental. The results from baking tests were: (1) Dough consistency appeared to decrease and dough-handling properties improved. This effect was greater in the case of II, since for I the amount of susceptible starch was a limiting factor, while II was not limited by the nature of the gluten substrate. (2) Bread properties such as the color of the crust, loaf volume, and crumb characteristics improved. Crumb compressibility at different storage times was determined by using a panimeter and this showed that softness of the bread had increased. Similarly carried out studies showed that, owing to the effect of I and II, the maltose value was raised only slightly while gas production, measured over a period of several hrs., was increased considerably, I being less effective than II. It is suggested that increased gas production, which becomes more pronounced under the action of heat during the first half of the baking process, contributes to better oven spring and improved loaf volume The maximum paste viscosity (measured with a Brabender Amylograph) was hardly affected by the fungal enzymes because of their low inactivation temps. Thus, it is

claimed that treatment with fungal enzymes permits the formation of sugars without any appreciable decrease in the viscosity of gelatinized starch. Also, the formation of dextrins at elevated temps. will be held at a min. and the choice of the enzyme level may be less critical than for malt α-amylase, which has a relatively high inactivation temperature Other suggested advantages are increased availability and mild degradation conditions of starch and liberation of bound β-amylase, which increase the rate of starch hydrolysis and gas production. The presence of other factors not included in this study, e.g. the quality of susceptible starch, nature of starch granules and gluten proteins, α-amylase content of flour, influence of proteolytic enzymes on bound enzymes, etc. may, of course, influence the response of the flour to fungal enzymes.

L8 ANSWER 20 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1951:48245 HCAPLUS
DOCUMENT NUMBER: 45:48245
ORIGINAL REFERENCE NO.: 45:8197b-d
TITLE: Utilization of fungal amylase for alcohol production
AUTHOR(S): Corman, Julian; Tsuchiya, H. M.
CORPORATE SOURCE: Northern Regional Research Lab., Peoria, IL
SOURCE: Cereal Chemistry (1951), 28, 280-8
CODEN: CECHAF; ISSN: 0009-0352
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB Alc. yield from grain mashes converted with fungal amylase is positively correlated with its maltase content if an adequate amount of α-amylase is present. The supplementation of fungal amylase containing moderate amts. of α-amylase and maltase with small amts. of malt does not spare the amount of fungal amylase required for satisfactory conversion of grain mashes, while the supplementation of fungal amylase high in maltase and low in α-amylase with small amts. of malt does lower the requirements of fungal amylase. Conversion of grain mashes at temps. as high as 65° is more advantageous than at lower temperature because more rapid liquefaction is effected. Fungal maltase is more thermostable than fungal .alpha.-
amylase which is the reverse of malt. The fungal amylase process is adaptable to the production of alc. from various farinaceous materials.

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=> e bisgard h/
E1            1     BISGALVINOXYLS/BI
E2            4     BISGARD/BI
E3            0 --> BISGARD H/BI
E4            1     BISGEMDICHLOROCYCLOPROPANE/BI
E5            1     BISGEMIFTALATA/BI
E6            1     BISGENTIOBIOSYL/BI
E7            1     BISGEOPROSPECTING/BI
E8            3     BISGERANYL/BI
E9            1     BISGERANYLOXYBIS/BI
E10           1     BISGERMAETHENE/BI
E11           1     BISGERMANDIYL/BI
E12           1     BISGERMANE/BI

=> e frantzen h/au
E1           35     FRANTZEN F/AU
E2           20     FRANTZEN FRANK/AU
E3            0 --> FRANTZEN H/AU
E4            3     FRANTZEN H B/AU
E5            1     FRANTZEN H P/AU
E6            1     FRANTZEN HANDELAND G/AU
E7            3     FRANTZEN HENRIK B/AU
E8           24     FRANTZEN I/AU
E9            2     FRANTZEN I M/AU
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E10           4      FRANTZEN INGE/AU
E11           2      FRANTZEN INGE MARJOLEIN/AU
E12          39      FRANTZEN J/AU

=> e bisgard f h/
E1            1      BISGALVINOXYLS/BI
E2            4      BISGARD/BI
E3            0 --> BISGARD F H/BI
E4            1      BISGEMDICHLOROCYCLOPROPANE/BI
E5            1      BISGEMIFTALATA/BI
E6            1      BISGENTIOBIOSYL/BI
E7            1      BISGEOPROSPECTING/BI
E8            3      BISGERANYL/BI
E9            1      BISGERANYLOXYBIS/BI
E10           1      BISGERMAETHENE/BI
E11           1      BISGERMANDIYL/BI
E12           1      BISGERMANE/BI

=> e svcensen a/au
E1            1      SVAZZI G/AU
E2            1      SVBODA H/AU
E3            0 --> SVCENSEN A/AU
E4            1      SVCHENIKOV N YU/AU
E5            1      SVCHENKO A F/AU
E6            1      SVCHENKOVA L V/AU
E7            1      SVCHILIN V A/AU
E8            1      SVCHUBERT F/AU
E9            1      SVCIK J/AU
E10           1      SVCJDA S/AU
E11           1      SVCTOZARSKII S V/AU
E12           1      SVD F H/AU

=> e svendsen a/au
E1            1      SVENDSE F/AU
E2            6      SVENDSEN/AU
E3          425 --> SVENDSEN A/AU
E4            1      SVENDSEN A A/AU
E5          363      SVENDSEN A B/AU
E6          109      SVENDSEN A BAERHEIM/AU
E7            1      SVENDSEN A BARHEIM/AU
E8           18      SVENDSEN A J/AU
E9           12      SVENDSEN A K/AU
E10           6      SVENDSEN A L/AU
E11           6      SVENDSEN A M/AU
E12           3      SVENDSEN A M B/AU

=> s e3
L9          425 "SVENDSEN A"/AU

=> e pedersen s/au
E1            1      PEDERSEN RUNE J S/AU
E2            1      PEDERSEN RUTH L/AU
E3         1413 --> PEDERSEN S/AU
E4            4      PEDERSEN S */AU
E5          561      PEDERSEN S A/AU
E6            7      PEDERSEN S A S/AU
E7            1      PEDERSEN S ANKER/AU
E8          430      PEDERSEN S B/AU
E9            1      PEDERSEN S BOEL/AU
E10          66      PEDERSEN S C/AU
E11          15      PEDERSEN S D/AU
E12           1      PEDERSEN S DIAMETER REN/AU

=> s e3
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L10        1413 "PEDERSEN S"/AU

=> s l9 or l10
L11        1836 L9 OR L10

=> d his

     (FILE 'HOME' ENTERED AT 09:28:24 ON 15 AUG 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:29:08 ON 15 AUG 2006
L1         54958 S ALPHA (W)AMYLASE?
L2        369714 S FUNGAL OR FUNGAMYL
L3           605 S L1 (A)L2
L4           249 S TERMOSTAB?
L5             0 S L3 AND L4
L6         72916 S THERMOSTAB?
L7            39 S L3 AND L6
L8            20 DUP REM L7 (19 DUPLICATES REMOVED)
                 E BISGARD H/
                 E FRANTZEN H/AU
                 E BISGARD F H/
                 E SVCENSEN A/AU
                 E SVENDSEN A/AU
L9           425 S E3
                 E PEDERSEN S/AU
L10         1413 S E3
L11         1836 S L9 OR L10

=> s l3 and l11
L12           7 L3 AND L11

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13           4 DUP REM L12 (3 DUPLICATES REMOVED)

=> d 1-4 ibib ab

L13   ANSWER 1 OF 4  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-10916  BIOTECHDS
TITLE:            Producing fungal alpha-amylase
                  variants which is useful for preparing dough or baked from
                  dough product, based on comparison of three-dimensional
                  structures of fungal alpha-
                  amylase and maltogenic alpha-amylase;
                     recombinant alpha-amylase production for use in food
                     industry
AUTHOR:           SVENDSEN A; BEIER L; VIND J; SPENDLER T; JENSEN M T
PATENT ASSIGNEE:  NOVOZYMES AS
PATENT INFO:      WO 2005019443 3 Mar 2005
APPLICATION INFO: WO 2004-DK558 23 Aug 2004
PRIORITY INFO:    DK 2003-1201 22 Aug 2003; DK 2003-1201 22 Aug 2003
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2005-202646 [21]
AB    DERWENT ABSTRACT:
      NOVELTY - Producing (M1) a variant polypeptide, by superimposing
      three-dimensional model of fungal alpha-
      amylase and for maltogenic alpha-amylase, selecting amino acid
      residue in fungal amylase with C-alpha atom located greater than
      0.8Angstrom from C-alpha atom of amino acid residue in maltogenic
      alpha-amylase and less than 11Angstrom from atom of enzyme substrate,
      altering the fungal amylase sequence, and producing the polypeptide
      having resulting amino acid sequence.
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DETAILED DESCRIPTION - Producing (M1) a variant polypeptide, involves providing an amino acid sequence and a three-dimensional model for a fungal alpha-amylase and for maltogenic alpha-amylase, where one or both models includes a substrate, superimposing the two three-dimensional models, selecting an amino acid residue in the fungal amylase which has C-alpha atom located greater than 0.8Angstrom from the C-alpha atom of any amino acid residue in the maltogenic alpha-amylase and less than 11Angstrom from an atom of a substrate, altering the fungal amylase sequence, where the alteration includes substitution or deletion of the selected residue or by insertion of a residue adjacent to the selected residue, and producing the polypeptide having the resulting amino acid sequence. An INDEPENDENT CLAIM is also included for a polypeptide (I) comprising (a) an amino acid sequence having at least 70% identity to a fully defined 478 amino acids (SEQ ID No:2) sequence given in the specification, and compared to SEQ ID No:2 comprises an amino acid alteration which is a deletion, substitution or insertion at a position corresponding to 15, 32-36, 63-64, 73-77, 119-120, 125-126, 151-152, 155-156, 167-172, 211 or 233-239, and has the ability to hydrolyze starch, (b) has an amino acid sequence having at least 70% identity to a fully defined 476 amino acids (SEQ ID No:3) sequence given in the specification, compared to SEQ ID No:3 comprises an amino acid alteration which comprises Q35K, Q35R, P70K, L151F, L151D, N233G+G234D, D75G, D75A or 166-171 (Glu-Gly-Asp-Thr-Ile-Val) substituted with Phe-Thr-Asp-Pro-Ala-Gly-Phe, and has the ability to hydrolyze starch, or (c) has an amino acid sequence having at least 70% identity to a fully defined 475 amino acids (SEQ ID No:4) sequence given in the specification, compared to SEQ ID No:4 comprises an amino acid alteration which comprises G35K, G35R, A76deletion+D77deletion, D74deletion+A78deletion, D74A, D74G, D77A, D77G, Y157W or L168F+A169T+T171P+P172A+T173G, and has the ability to hydrolyze starch.

BIOTECHNOLOGY - Preferred Method: In (M1), the substitution or insertion is made with an amino acid residue of the same type as the corresponding residue in the maltogenic alpha-amylase sequence, where the type is positively charged, negatively charged, hydrophilic or hydrophobic. The substitution or insertion is made with a larger or smaller amino acid residue depending on whether the corresponding residue in the maltogenic alpha-amylase sequence is larger or smaller. The alteration of the amino acid sequence further comprises substitution of a fungal alpha-amylase residue which has a C-alpha atom located less than 11Angstrom from an atom of a substrate and less than 0.8Angstrom from the C-alpha atom of a maltogenic alpha-amylase residue. The substitution is made with an amino acid residue of the same type as the corresponding maltogenic alpha-amylase residue, where the type is positive, negative, hydrophilic or hydrophobic. Preferred Polypeptide: In (I), the alteration corresponding to Q35K/R, Y75A/F, Y155W, L166F, G167T, N169P, T170A, L232Y, D233G, G234D, Y252F, Y256T, 166Leu-Gly-Asp-Asn-Thr-Val171 to Phe-Thr-Asp-Pro-Ala-Gly-Phe, 168-171 (Asp-Asn-Thr-Val) substituted with Asp-Pro-Ala-Gly-Phe, 168-171 (Asp-Asn-Thr-Val) substituted with Asp-Pro-Ala-Gly-Leu, 168-171 (Asp-Asn-Thr-Val) substituted with Asp-Pro-Ala-Gly-Cys, D233G+G234D, Q35K+Y75F+D168Y, Q35R+Y75F, Q35R+Y75F+D168Y, 168-171 (Asp-Asn-Thr-Val) substituted with Asp-Pro-Ala-Gly-Phe+Y75A, 168-171 (Asp-Asn-Thr-Val) substituted with Asp-Pro-Ala-Gly-Phe+Q35K+Y75A, 168-171 (Asp-Asn-Thr-Val) substituted with Asp-Pro-Ala-Gly-Phe+Q35K+Y75A+D233G+G234D, 168-171 (Asp-Asn-Thr-Val) substituted with Asp-Pro-Ala-Gly-Phe+Y75A+G234D, 168-171 (Asp-Asn-Thr-Val) substituted with Asp-Pro-Ala-Gly-Phe+Y75A+D233G+G234D, 166-171 (Leu-Gly-Asp-Asn-Thr-Val) substituted with Phe-Thr-Asp-Pro-Ala-Gly-Phe+Y75A, 166-171 (Leu-Gly-Asp-Asn-Thr-Val) substituted with Phe-Thr-Asp-Pro-Ala-Gly-Phe+Q35K+Y75A and 166-171 (Leu-Gly-Asp-Asn-Thr-Val) substituted with Phe-Thr-Asp-Pro-Ala-Gly-Phe+Q35K+Y75A+D233G+G234D.

USE - (M1) is useful for producing a variant polypeptide. The polypeptide produced by (M1) is useful for preparing a dough or a baked from dough product (all claimed). The polypeptide of (M1) is useful for

anti-staling in baked products.

ADVANTAGE - The variant polypeptide has improved anti-staling effect and higher degree of exo-amylase activity. (26 pages)

L13 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-04822 BIOTECHDS
TITLE: Producing soluble starch hydrolysate comprises subjecting aqueous granular starch slurry below initial gelatinization temperature of granular starch to action of Glycoside Hydrolase Family13 enzyme, and fungal amylase; fungus alpha-amylase, beta-amylase or glucoamylase-catalyzed starch hydrolysis for use in high fructose starch-based syrup, ethanol or sweetener production
AUTHOR: VIKSOE-NIELSEN A; ANDERSEN C; PEDERSEN S; HJORT C
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2004113551 29 Dec 2004
APPLICATION INFO: WO 2004-DK456 25 Jun 2004
PRIORITY INFO: DK 2003-1568 24 Oct 2003; DK 2003-949 25 Jun 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-075255 [08]
AB DERWENT ABSTRACT:

NOVELTY - Producing (M1) soluble starch hydrolysate, by subjecting aqueous granular starch slurry to action of first and second enzyme, where first enzyme is member of Glycoside Hydrolase Family13, having alpha-1,4-glucosidic hydrolysis activity and comprising functional carbohydrate-binding module; and second enzyme is a fungal alpha-amylase, beta-amylase or glucoamylase.

DETAILED DESCRIPTION - Producing (M1) a soluble starch hydrolysate, involves subjecting an aqueous granular starch slurry at a temperature below the initial gelatinization temperature of the granular starch to the action of a first enzyme and a second enzyme, where the first enzyme is a member of the Glycoside Hydrolase Family13, has alpha-1,4-glucosidic hydrolysis activity, and comprises a functional carbohydrate-binding module (CBM) belonging to CBM Family 20 having an amino acid sequence having at least 60% homology to a fully defined sequence of 102, 99 or 102 amino acids (S1) as given in the specification, and where the second enzyme is chosen from a fungal alpha-amylase (EC 3.2.1.1), beta-amylase (E.C. 3.2.1.2), and a glucoamylase (E.C. 3.2.1.3). INDEPENDENT CLAIMS are also included for the following: (1) a process (M2) for production of high fructose starch-based syrup (HFSS), where a soluble starch hydrolysate produced by (M1) is subjected to conversion into HFSS, such as high fructose corn syrup (HFCS); (2) process (M3) for production of a fermentation product, where a soluble starch hydrolysate produced by (M1) is subjected to fermentation into a fermentation product, such as citric acid, monosodium glutamate, gluconic acid, sodium gluconate, calcium gluconate, potassium gluconate, glucono delta lactone, sodium erythorbate, itaconic acid, lactic acid, gluconic acid, ketones, amino acids, glutamic acid (sodium monoglutaminate), penicillin, tetracycline, enzymes, vitamins, such as riboflavin, B12, beta-carotene or hormones; (3) a process (M4) for production of fuel or potable ethanol, where a soluble starch hydrolysate produced by (M1) is subjected to fermentation into ethanol; (4) use of an enzyme (I) having alpha-amylase activity in a process for hydrolysis of starch, the enzyme comprising a functional CBM having an amino acid sequence having at least 60% homology to (S1), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 98%, such as at least 99% homology to an amino acid sequence chosen from a fully defined sequence of 619, 613, 619 and 640 amino acids (S2) as given in the specification; and (5) use of an enzyme having alpha-amylase activity in a process for hydrolysis of granular starch, the enzyme comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 98%, such as at least 99% homology to an amino acid sequence chosen from a fully defined sequence of 484, 485, 484, 517, 550, 482, 482, 482, 482, 483, 483, 483, 485, 484, 485 amino acids (S3) as

given in the specification.

BIOTECHNOLOGY - Preferred Method: In (M1), the alpha-amylase comprises a functional CBM having at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, such as at least 99% homology to (S1) or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 98%, such as at least 99% homology to (S2) or (S3). The starch slurry has 20-55% dry solids granular starch, preferably 25-40% dry solids granular starch, more preferably 30-35% dry solids, especially around 33% dry solids granular starch. In (M1), at least 85-98%, preferably 99% of the dry solids of the granular starch are converted into a soluble starch hydrolysate. (M1) involves subjecting the granular starch slurry to the action of an isoamylase and/or pullulanase. The temperature is at least 58 degrees C, 59 degrees C, preferably 60 degrees C. The pH is 3.0-7.0, preferably 3.5-6.0, more preferably 4.0-5.0. The soluble starch hydrolysate has a DX of at least 94.5%, 95.0%, 95.5%, 96.0%, 96.5%, 97.0%, 97.5%, 98.0%, 98.5%, 99.0% or at least 99.5%. . (M1) is conducted in an ultrafiltration system, or in a continuous membrane reactor with ultrafiltration or microfiltration membranes, and where the retentate is held under recirculation in presence of enzymes, raw starch and water, where the permeate is the soluble starch hydrolysate. (M1-M4) are conducted in an ultrafiltration system or in a continuous membrane reactor with ultrafiltration membranes, where the retentate is held under recirculation in the presence of enzymes, raw starch, yeast, yeast nutrients and water and the permeate is an ethanol containing liquid. The starch slurry is being contacted with a polypeptide comprising a CBM, but no catalytic module, that is a loose CBM. In (M4), the fermentation step is carried out simultaneously or separately/sequential to the hydrolysis of the granular starch. Preferred Components: The granular starch is obtained from tubers, roots, stems, whole grain, cereals, corn, cobs, wheat, barley, rye, milo, sago, cassava, tapioca, sorghum, rice or potatoes, or from dry milling of whole grain, wet milling of whole grain, or milled corn grits

USE - (M1) is useful for producing a soluble starch hydrolysate which is useful for production of high fructose starch-based syrup (HFSS), a fermentation product, fuel or potable ethanol. (I) is useful for hydrolysis of granular starch (claimed). The hydrolysates are useful as sweeteners or as precursors for other saccharides, such as fructose.

EXAMPLE - A slurry with 33% dry solids (DS) granular starch was prepared by adding 247.5 g of wheat starch under stirring to 502.5 ml of water. The pH was adjusted with HCl to 4.5. The granular starch slurry was distributed to 100 ml blue cap flasks with 75 g in each flask. The flasks were incubated with magnetic stirring in a 60 degrees C water bath. At zero hours the enzyme activities were dosed to the flasks. Samples were withdrawn after 24, 48, 72, and 96 hours. The starch was completely hydrolyzed by adding an excess amount of alpha-amylase (300 KNU/Kg dry solids) and placing the sample in an oil bath at 95 degrees C for 45 minutes. Subsequently the samples were cooled to 60 degrees C and an excess amount of glucoamylase (600 AGU/kg DS) was added followed by incubation for 2 hours at 60 degrees C. Soluble dry solids in the starch hydrolysate were determined by refractive index measurement on samples after filtering through a 0.22 microM filter. The sugar profile was determined by HPLC. The amount of glucose was calculated as DX. The amount of the soluble hydrolysate obtained after 24, 48, 72 and 96 hours were 88.4, 92.4, 93.7 and 95.3, respectively. (68 pages)

L13 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-04043 BIOTECHDS
TITLE: Production of fermentation product, e.g. ethanol, involves carrying out a fermentation step with at least one carbohydrate-source generating enzyme activity and at least one alpha-amylase activity;
with use of Bacillus stearothermophilus, Aspergillus niger, Talaromyces emersonii or Rhizomucor miehei alpha-amylase

AUTHOR: OLSEN H S; PEDERSEN S; BECKERICH R; VEIT C; FELBY C
PATENT ASSIGNEE: NOVOZYMES AS; NOVOZYMES NORTH AMERICA INC
PATENT INFO: WO 2002074895 26 Sep 2002
APPLICATION INFO: WO 2002-DK179 19 Mar 2002
PRIORITY INFO: US 2001-304380 10 Jul 2001; US 2001-277383 19 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-723447 [78]

AB DERWENT ABSTRACT:

NOVELTY - A fermentation product is produced by carrying out a fermentation step in the presence of at least one carbohydrate-source generating enzyme activity and at least one alpha-amylase activity.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (a) A composition comprising: (i) carbohydrate-source generating enzyme activity; (ii) alpha-amylase activity, protease activity; and (iii) debranching enzyme activity; and (b) Use of the composition for saccharification and/or fermentation, for ethanol production or for beer or wine production.

BIOTECHNOLOGY - Preferred Component: The carbohydrate-source generating enzyme is a glucoamylase particularly derived from Aspergillus niger or Talaromyces emersonii, beta-amylase particularly derived from barley or a maltogenic amylase particularly derived from Bacillus stearothermophilus. The alpha-amylase is an acid alpha-amylase, particularly an acid fungal alpha-amylase, e.g. an acid fungal alpha-amylase derived from A. niger or A. oryzae. The ratio between the acid fungal alpha-amylase activity (AFAU) per glucoamylase activity (AGU) (AFAU per AU) is at least0.1 (particularly at least0.16), preferably 0.12-0.3. The protease is an acid protease, particularly an acid fungal protease, e.g. an acid fungal protease derived from a strain of Aspergillus (particularly A. niger or A. oryzae) or a strain of Rhizomucor (particularly R. miehei) or a bacterial protease, e.g. acid, neutral or alkaline protease, (e.g., a protease derived from a strain of Bacillus) particularly ALCALASE or NEUTRASE. The debranching enzyme is an isoamylase (E.C. 3.2.1.68) or pullulanase (E.C. 3.2.1.41), particularly a pullulanase derived from Bacillus sp., e.g. a strain of B. deramificans, B. acidopullulyticus or B. naganoensis. The glucoamylase/pullulanase ratio determined as AGU/PUN is 5:1-1:5. The micro-organism is a yeast, e.g. a yeast belonging to Saccharomyces spp. (particularly Saccharomyces cerevisae). The material to be fermented is a liquefied whole grain mash or a side stream from starch processing, particularly liquefied starch with a DE of 8-10. The yeast cell wall degrading enzyme is a preparation, e.g. the product GLUCANEX (RTM: enzyme) derived from Trichoderma harzianum.

USE - The inventive process is used for producing a fermentation product, preferably ethanol, beer or wine. The produced ethanol can be used as fuel ethanol, drinking ethanol (i.e., potable neutral spirits) or industrial alcohol. The grain, a left-over from the fermentation or distillation steps, is typically used for animal feed either in liquid or dried form.

ADVANTAGE - The inventive method results in increased fermentation rate and ethanol yield.

EXAMPLE - Washed yeast (2.5 g) was suspended in ion-exchanged water (100 mL) at room temperature. The suspension was stirred on a magnetic stirrer for 15 minutes. Samples (15 mL) were transferred to centrifuge tubes with volume indication. Sodium chloride (NaCl), calcium chloride (CaCl2) and Rhizomucor miehei protease was added to create the solutions of 250 mM NaCl, 4 mM calcium chloride (CaCl2) and 4 mM CaCl2 and R. miehei protease. Incubation of the solutions was made at room temperature for 15 minutes in a rotary shaker, which turned the closed tubes end-over-end at 20 rpm. The tubes were left in vertical position for 60 minutes after which the volume of the sediment was measured. The results showed the effect of R. miehei protease on volume of sediment. For the solution containing 250 mM NaCl the volume of sediment was 0.165 mL, and

for the solution containing 4 mM CaCl2 the volume of sediment was 0.245 mL and for the solution containing 4 mM CaCl2 and R. miehei protease the volume of sediment was 0.194 mL.(38 pages)

L13 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2000417503 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10924103
TITLE: Structural analysis of a chimeric bacterial alpha-amylase. High-resolution analysis of native and ligand complexes.
AUTHOR: Brzozowski A M; Lawson D M; Turkenburg J P; Bisgaard-Frantzen H; Svendsen A; Borchert T V; Dauter Z; Wilson K S; Davies G J
CORPORATE SOURCE: Department of Chemistry, Structural Biology Laboratory, University of York, Heslington, UK.
SOURCE: Biochemistry, (2000 Aug 8) Vol. 39, No. 31, pp. 9099-107. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1E3X; PDB-1E3Z; PDB-1E43; PDB-1E4PHI
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 15 Sep 2000
Last Updated on STN: 22 Sep 2000
Entered Medline: 7 Sep 2000

AB Several chimeric alpha-amylases genes were constructed by an in vivo recombination technique from the Bacillus amyloliquefaciens and Bacillus licheniformis genes. One of the fusion amylases (hereafter BA2), consisting of residues 1-300 from B. amyloliquefaciens and 301-483 from B. licheniformis, has been extensively studied by X-ray crystallography at resolutions between 2.2 and 1.7 A. The 3-dimensional structure of the native enzyme was solved by multiple isomorphous replacement, and refined at a resolution of 1.7 A. It consists of 483 amino acids, organized similarly to the known B. lichiniformis alpha-amylase structure [Machius et al. (1995) J. Mol. Biol. 246, 545-559], but features 4 bound calcium ions. Two of these form part of a linear cluster of three ions, the central ion being attributed to sodium. This cluster lies at the junction of the A and B domains with one calcium of the cluster structurally equivalent to the major Ca(2+) binding site of fungal alpha-amylases. The third calcium ion is found at the interface of the A and C domains. BA2 contains a fourth calcium site, not observed in the B. licheniformis alpha-amylase structure. It is found on the C domain where it bridges the two beta-sheets. Three acid residues (Glu261, Asp328, and Asp231) form an active site similar to that seen in other amylases. In the presence of TRIS buffer, a single molecule of TRIS occupies the -1 subsite of the enzyme where it is coordinated by the three active-center carboxylates. Kinetic data reveal that BA2 displays properties intermediate to those of its parents. Data for crystals soaked in maltooligosaccharides reveal the presence of a maltotriose binding site on the N-terminal face of the (beta/alpha)(8) barrel of the molecule, not previously described for any alpha-amylase structure, the biological function of which is unclear. Data for a complex soaked with the tetrasaccharide inhibitor acarbose, at 1.9 A, reveal a decasaccharide moiety, spanning the -7 to +3 subsites of the enzyme. The unambiguous presence of three unsaturated rings in the (2)H(3) half-chair/(2)E envelope conformation, adjacent to three 6-deoxypyranose units, clearly demonstrates synthesis of this acarbose-derived decasaccharide by a two-step transglycosylation mechanism.

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(FILE 'HOME' ENTERED AT 09:28:24 ON 15 AUG 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 09:29:08 ON 15 AUG 2006

L1	54958	S	ALPHA (W)AMYLASE?
L2	369714	S	FUNGAL OR FUNGAMYL
L3	605	S	L1 (A)L2
L4	249	S	TERMOSTAB?
L5	0	S	L3 AND L4
L6	72916	S	THERMOSTAB?
L7	39	S	L3 AND L6
L8	20	DUP REM	L7 (19 DUPLICATES REMOVED)
		E	BISGARD H/
		E	FRANTZEN H/AU
		E	BISGARD F H/
		E	SVCENSEN A/AU
		E	SVENDSEN A/AU
L9	425	S	E3
		E	PEDERSEN S/AU
L10	1413	S	E3
L11	1836	S	L9 OR L10
L12	7	S	L3 AND L11
L13	4	DUP REM	L12 (3 DUPLICATES REMOVED)

	L #	Hits	Search Text
1	L1	1	"7005288".pn.
2	L2	9959	alpha adj amylase$2
3	L3	54873	fungal or fungamyl
4	L4	1110	l2 same l3
5	L5	12023	thermostabl?
6	L6	22	l4 same l5
7	L7	12599	BISGARD-FRANTZEN-HENRIK SVENDSEN PEDERSEN
8	L8	188	l4 and l7
9	L9	56	l5 and l8

	Issue Date	Pages	Document ID	Title
1	20060316	9	US 20060057270 A1	Flour based food product comprising thermostable alpha-amylase
2	20060309	34	US 20060051849 A1	Thermostable alpha-amylases
3	20051124	21	US 20050260719 A1	Method of producing saccharide preparations
4	20051027	13	US 20050239158 A1	Enzyme-based monitoring device for thermal processing of objects
5	20050210	21	US 20050031734 A1	Grain compositions containing pre-biotic isomalto-oligosaccharides and methods of making and using same
6	20050203	17	US 20050026261 A1	Ethanol production by simultaneous saccharification and fermentation (SSF)
7	20041118	17	US 20040229764 A1	Fungamyl-like alpha-amylase variants
8	20040617	12	US 20040112559 A1	Paper-making or non paper-making use of a starchy composition containing a selected cationic starchy material
9	20040513	15	US 20040091983 A1	Secondary liquefaction in ethanol production
10	20030327	14	US 20030059901 A1	Process for isomaltose production
11	20021107	23	US 20020164723 A1	Method of producing saccharide preparations

12	20060228	16	US 7005288 B1	Fungamyl-like alpha-amylase variants

	Issue Date	Pages	Document ID	Title
13	20011211	12	US 6329182 B1	Method of producing oligosaccharide syrups, a system for producing the same and oligosaccharide syrups
14	20011016	19	US 6303346 B1	Method of producing saccharide preparations
15	20001024	15	US 6136571 A	Method of producing saccharide preparations
16	20001010	19	US 6129788 A	Method of producing saccharide preparations
17	20000215	14	US 6025168 A	Method for the production of isomalto-oligosaccharide rich syrups
18	19900918	8	US 4957563 A	Starch conversion
19	19880105	11	US 4717662 A	Thermal stabilization of alpha-amylase
20	19851224	15	US 4560651 A	Debranching enzyme product, preparation and use thereof
21	19831122	18	US 4416903 A	Antistaling baking composition
22	19820316	13	US 4320151 A	Antistaling baking composition

	Issue Date	Pages	Document ID	Title
1	20060615	22	US 20060127889 A1	Method for assessing transgene expression and copy number
2	20060406	78	US 20060073583 A1	Polypeptide
3	20060330	74	US 20060068406 A1	Single-stranded nucleic acid template-mediated recombination and nucleic acid fragment isolation
4	20060126	63	US 20060018997 A1	Polypeptide
5	20060112	55	US 20060008890 A1	Polypeptide
6	20060112	54	US 20060008888 A1	Polypeptide
7	20051229	141	US 20050287537 A1	Pullulanase variants and methods for preparing such variants with predetermined properties
8	20051124	21	US 20050260719 A1	Method of producing saccharide preparations
9	20051117	36	US 20050255574 A1	Proteins
10	20050915	31	US 20050202533 A1	Maltogenic alpha-amylase variants
11	20050804	59	US 20050170487 A1	Alpha-amylase mutants
12	20050602	53	US 20050118695 A1	Alpha-amylase mutants
13	20050526	110	US 20050112237 A1	Polypeptide

14	20050303	18	US 20050048636 A1	Glucoamylase variants

	Issue Date	Pages	Document ID	Title
15	20050217	70	US 20050037391 A1	Polypeptide
16	20050127	58	US 20050019886 A1	Alpha-amylase variants
17	20041216	74	US 20040253671 A1	Method
18	20041118	17	US 20040229764 A1	Fungamyl-like alpha-amylase variants
19	20041104	22	US 20040219649 A1	Alcohol product processes
20	20040617	17	US 20040115779 A1	Fermentation process
21	20040429	137	US 20040082028 A1	Pullulanase variants and methods for preparing such variants with predetermined properties
22	20040311	52	US 20040048351 A1	Alpha-amylase mutants
23	20040311	128	US 20040048247 A1	Pullulanase variants and methods for preparing such variants with predetermined properties
24	20040101	30	US 20040002142 A1	Glucoamylase variants
25	20030911	50	US 20030171236 A1	ALPHA-AMYLASE MUTANTS
26	20030911	64	US 20030170769 A1	Alpha-amylase mutants
27	20030327	14	US 20030059901 A1	Process for isomaltose production

28	20030306	37	US 20030044954 A1	Alpha-amylase variants

	Issue Date	Pages	Document ID	Title
29	20021107	23	US 20020164723 A1	Method of producing saccharide preparations
30	20020905	17	US 20020123123 A1	Cutinase variants
31	20060425	39	US 7033627 B2	Production of enzymes in seeds and their use
32	20060228	16	US 7005288 B1	Fungamyl-like alpha-amylase variants
33	20051101	14	US 6960459 B2	Cutinase variants
34	20050405	112	US 6876932 B1	Maltogenic alpha-amylase variants
35	20050104	37	US 6838257 B2	Pullulanase variants and methods for preparing such variants with predetermined properties
36	20041109	46	US 6815190 B1	Cutinase variants
37	20031104	45	US 6642044 B2	.alpha.-amylase mutants
38	20030325	53	US 6537792 B1	Protein engineering of glucoamylase to increase pH optimum, substrate specificity and thermostability
39	20020827	99	US 6440716 B1	.alpha.-amylase mutants
40	20020820	46	US 6436888 B1	.alpha.-amylase mutants
41	20020625	34	US 6410295 B1	Alpha-amylase variants
42	20020305	43	US 6352851 B1	Glucoamylase variants

43	20020226	82	US 6350599 B1	Pullulanase variants and methods for preparing such variants with predetermined properties

	Issue Date	Pages	Document ID	Title
44	20011211	23	US 6329186 B1	Glucoamylases with N-terminal extensions
45	20011211	12	US 6329182 B1	Method of producing oligosaccharide syrups, a system for producing the same and oligosaccharide syrups
46	20011016	19	US 6303346 B1	Method of producing saccharide preparations
47	20010403	39	US 6211134 B1	Mutant .alpha.-amylase
48	20001226	20	US 6165770 A	Alkaline stable amylase from Thermoalcalibacter
49	20001219	97	US 6162628 A	Maltogenic alpha-amylase variants
50	20001107	47	US 6143708 A	.alpha.-amylase mutants
51	20001024	15	US 6136571 A	Method of producing saccharide preparations
52	20001010	19	US 6129788 A	Method of producing saccharide preparations
53	20000627	29	US 6080568 A	Mutant .alpha.-amylase comprising modification at residues corresponding to A210, H405 and/or T412 in Bacillus licheniformis
54	20000208	100	US 6022724 A	.alpha.-amylase mutants
55	19991123	100	US 5989169 A	.alpha.-amylase mutants
56	19990928	50	US 5958739 A	Mutant .alpha.-amylase